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Developmental Toxicity of *Citrus aurantium* in Rats

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Abstract

BACKGROUND—Ephedra was commonly used in herbal products marketed for weight loss until safety concerns forced its removal from products. Even before the ban, manufacturers had begun to replace ephedra with other compounds, including *Citrus aurantium*, or bitter orange. The major component in the bitter orange extract is synephrine which is chemically similar to ephedrine. The purpose of this study was to determine if relatively pure synephrine or synephrine present as a constituent of a bitter orange extract produced developmental toxicity in rats.

METHOD—Sprague-Dawley rats were dosed daily by gavage with one of several different doses of synephrine from one of two different extracts. Caffeine was added to some doses. Animals were sacrificed on GD 21, and fetuses were examined for the presence of various developmental toxic endpoints.

RESULTS AND CONCLUSION—At doses up to 100 mg synephrine/kg body weight, there were no adverse effects on embryoletality, fetal weight or incidences of gross, visceral or skeletal abnormalities. There was a decrease in maternal weight at 50 mg synephrine/kg body weight when given as the 6% synephrine extract with 25 mg caffeine/kg body weight; there was also a decrease in maternal weight in the caffeine only group. This decrease in body weight may have been due to decreased food consumption which was also observed in these two groups. Overall, doses of up to 100 mg synephrine/kg body weight did not produce developmental toxicity in Sprague-Dawley rats.

Keywords

bitter orange; *Citrus aurantium*; synephrine; birth defects; rats; ephedra; dietary supplement

Introduction

For a number of years, ephedra (Ma-Huang) was used in dietary supplements marketed for weight loss and energy. These products were noted to have significant adverse effects (Haller and Benowitz, 2000; Shekelle et al., 2003). In April 2004, the Food and Drug Administration banned the use of ephedra at concentrations of more than 10 mg in products. Manufacturers had already begun to use extracts of *Citrus aurantium* (also known as bitter orange, Seville orange, or zhi shi) as a replacement for ephedra (Marcus and Grollman,

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2003). Most of these extracts are standardized to 4–6% synephrine. The peel of this immature bitter orange has been used in traditional Chinese medicine for relief of indigestion, abdominal pain, constipation, and dysenteric diarrhea. A recent survey in Charleston, SC found that *Citrus aurantium* was among the top ten ingredients in non-prescription weight loss supplements (Sharpe et al., 2006).

The use of dietary supplements for weight loss is fairly common in the United States. Surveys have found that 15.2% (Blanck et al., 2007) to 33.9% (Pillitteri et al., 2008) of adults admit that they have at some time used a dietary supplement to aid in weight loss. In most cases, the individuals did not discuss this use with their physicians. Two short-term trials have suggested that these supplements may help in weight loss (Colker et al., 1999; Kalman et al., 2000), but constituents other than *Citrus aurantium* extract were present in the products used in these studies. Therefore, it remains unclear if products containing *Citrus aurantium* extracts are efficacious in promoting weight loss.

Although dietary supplements generally include a warning that they not be used during pregnancy, there is little to no data on the safety of these constituents when used by pregnant women. Recent surveys have indicated that 5.8% (Louik et al., 2010) to 10.9% (Broussard et al., 2010) of women have reported using herbal treatments prior to or during pregnancy. The survey by Louik et al. (2010) included interviews from 1998–2006 when 1.1% of 4866 women surveyed had used herbal products for weight loss or sports enhancement; Metabolife, an ephedra-containing product, was the most commonly mentioned product in this group. Ephedra-containing products were also among the most commonly taken herbals in the survey by Broussard et al. (2010) which covered 1998–2004. In this survey, 1.2% of 4239 women had taken ephedra-containing products either before or during their pregnancy. Bitsko et al. (2008) using data from the National Birth Defects Prevention Study found an increased risk of anencephaly, dextro-transposition of the great arteries or aortic stenosis associated with the use of weight loss containing products during pregnancy. An increased odds ratio for anencephaly was observed among women taking ephedra-containing products; other weight loss products were associated with increased odds ratios for dextro-transposition of the great arteries and aortic stenosis.

The chemical composition of *Citrus aurantium* extracts is complex. The orange peel contains flavones, carotenoids and alkaloids such as synephrine (4-hydroxy- α -[(methylamino)methyl]-benzenemethanol; CAS 94-07-5), octopamine (α -(aminomethyl)-4-hydroxybenzenemethanol; CAS 104-14-3), hordenine (*N,N*-dimethyltyramine; CAS 539-15-1) and tyramine (CAS 51-67-2). Synephrine is present in nearly all citrus products and is naturally consumed by humans in small amounts if citrus is included in the diet. Structurally, synephrine is similar to ephedrine (Fig. 1); it is also thought to have similar biological activity and may provide an energy boost, suppress appetite and increase metabolic rate and caloric expenditure. However, as with ephedra-containing supplements, the safety and efficacy of products containing *Citrus aurantium* extract have been questioned (Fugh-Berman and Myers, 2004; Bent et al., 2004). The suggested *Citrus aurantium* daily dose is 36–180 mg/day taken as a divided dose two to three times per day.

Labeling of synephrine-containing supplements may be somewhat unreliable. Niemann and Gay (2003) observed that only 6/14 products that they analyzed contained an amount of synephrine within 20% of the labeled amount; 2/14 contained no synephrine, 3/14 contained 120–135% of the labeled amount, with the remaining 3 products containing significantly less than the labeled amount of synephrine. Evans and Siitonen (2008) evaluated the alkaloid content of twenty supplements which supposedly contained bitter orange extract. They did not compare the level of synephrine to that specified in the label, but they observed

a ten-fold range of concentrations of synephrine among the products, and three of the supplements contained no detectable synephrine.

Nearly all of the *Citrus aurantium*-containing supplements also contain caffeine, either from plant sources or anhydrous caffeine. Evans and Siitonen (2008) evaluated the caffeine contents of twenty bitter orange supplements and found a nearly ten-fold range in concentration. Using the manufacturer's recommended daily dose, these supplements would provide 125–728 mg of caffeine. It has been estimated that food and beverages provide 106–170 mg caffeine/day (Knight et al., 2004) or 193 mg caffeine/day (Frary et al., 2005), suggesting that these supplements could be a major source of caffeine for some individuals.

To date there is only a single report of teratogenicity of synephrine in rats. When administered daily on GD 6 – 15 by intramuscular injection at 55 or 110 mg/kg, synephrine was found to produce retardation in skeletal ossification, an increase in brain defects, and hemorrhagic injuries (Scrollini et al., 1970, cited by Schardein, 2000). There is a report of an increase of situs inversus in the heart and/or gut of *Xenopus laevis* embryos exposed to octopamine, an alkaloid also found in *Citrus aurantium* extracts (Toyoizumi et al., 1997). These reports suggest that compounds such as synephrine and octopamine may represent a risk for developmental abnormalities. In addition, many weight loss supplements contain a natural source of caffeine, and caffeine has been shown to enhance the developmental toxicity of ephedrine in chicks (Nishikawa et al., 1985a). Therefore, the purpose of this study was to determine the developmental toxicity potential of *Citrus aurantium* extracts alone and in combination with caffeine in rats.

Materials and Methods

Chemicals

Extracts containing either 6% synephrine (also referred to as bitter orange or BO) or 90% synephrine (also referred to as synephrine or SE) were purchased from Modern Nutrition and Biotech (Appleton, WI). Both extracts were characterized by HPLC with photodiode array detection with separation compared to that of known standards ((±)-synephrine (min. 99.5% pure), tyramine hydrochloride (min. 99.5% pure), and octopamine hydrochloride (99.55% pure) obtained from Sigma Chemical Co. (St. Louis, MO); hordenine sulfate (98.0% pure) obtained from TCI America (Portland, OR), and N-methyltyramine graciously supplied by Dr. Iklas A. Khan from the National Center for Natural Products Research at the University of Mississippi). Further identity was confirmed by HPLC/MS and GC/MS analysis. These analyses demonstrated that the BO extract contained 7.25% synephrine, 0.63% hordenine, 0.10% octopamine and 0.09% tyramine by weight. The SE extract contained 95.0% synephrine, 0.05% hordenine, 0.39% octopamine, and 0.02% tyramine by weight. Both extracts were tested by the Arkansas Regional Laboratory ORA lab and found by GC/FPD and GC/MS analyses to contain no detectable levels of pesticides. Microbiological assay revealed no contamination of the extracts. Anhydrous caffeine was obtained from Sigma Chemical Co. (St. Louis, MO).

Animals and animal care

Adult female Sprague-Dawley rats from the FDA's National Center for Toxicological Research (NCTR) colony were used in this study. All animals were housed individually in plastic cages with a small amount of hardwood chip bedding. Food (NIH-31) and tap water were available *ad libitum*. Temperature ($23 \pm 3^{\circ}\text{C}$), relative humidity ($50 \pm 20\%$) were monitored and held constant; lights were on from 7 AM to 7 PM daily. Food consumption was monitored weekly, and the animals were weighed daily just prior to gavage dosing at approximately 9 AM.

All animal procedures were approved by the NCTR Institutional Animal Care and Use Committee and followed the “National Research Council: Guide for the Care and Use of Laboratory Animals” (NRC, 1996).

Experimental design

A dose-finding study was done with groups of seven rats. Animals were bred overnight, and the morning that a vaginal sperm plug was found was considered GD 0. Rats were dosed daily by gavage from GD 3 until GD 20 and were sacrificed on GD 21. Eight dose groups were examined: vehicle (0.25% methyl cellulose); 1.0, 2.5, 5.0, 10.0 and 25.0 mg/kg body weight using the SE extract; 10.0 and 25.0 mg/kg body weight using the BO extract. It is important to note that the doses indicated above are the doses of the alkaloid synephrine that were administered; the source of synephrine was either the 95% extract (SE) or the 6% extract (BO). Both extracts were dosed by gavage at 5 ml/kg body weight. Before each dosing solution was used, it was analyzed and certified to be $\pm 10\%$ of the target dose. Dosing solutions were stored at room temperature in amber bottles with constant stirring; they were stable under these conditions for at least 14 days.

Animals were sacrificed by over-exposure to carbon dioxide. Blood was removed from the dam by cardiac puncture. Various maternal organs were removed and weighed; these included the liver, brain, heart, kidneys, adrenal glands and thymus. The uterus was removed, weighed and the contents were examined. The status of each implantation site was noted. Each live fetus was weighed, examined for external abnormalities, anesthetized and decapitated. Each fetus was then examined for internal abnormalities according to Stuckhardt and Poppe (1984); several fetal organs [lungs, heart, liver, kidneys, spleen, adrenals and thymus] were removed and weighed. Skeletal structures were stained and evaluated according to LaBorde et al. (1995).

Based on the results of the dose-finding experiment, a full teratology study was then conducted. Because none of the doses in the dose-finding study produced developmental toxicity, higher doses were used in the full study. Adult female Sprague-Dawley rats from the NCTR colony were again used. The day that the sperm plug was found was considered GD 0; rats were gavage dosed daily from GD 3 to GD 20. Groups of 25 rats were treated with one of nine doses. These doses were vehicle (0.25% methyl cellulose); 10, 25, 50 or 100 mg of synephrine/kg body weight (using the BO extract), 50 and 100 mg synephrine/kg body weight (using the SE extract); 50 mg synephrine/kg body weight + 25 mg caffeine/kg body weight (using the SE extract), and 25 mg caffeine/kg body weight. Maternal body weight was determined daily just prior to dosing, and food consumption was determined weekly. All animals were sacrificed on GD 21 by over-exposure to carbon dioxide. Uterine removal and fetal examination were done as described for the dose-finding study.

Statistics

Tests were conducted as two-sided at the 0.05 level of significance. Means and standard deviations for each treatment group were calculated using formulas in Excel. Statistical analyses for the teratology study were performed using SAS 9.2 (SAS Institute).

Dose-Finding Study

In order to compare each treatment to the control group, an F-test was done to determine if the variances were equal. A Student's two-sided t-test was then done to determine differences between treatment and control groups. All tests were calculated with formulas in Excel.

Teratology Study

Contrasts were performed to compare treatment groups to the vehicle control group and between treatment groups using Holm's method of adjustment for multiple comparisons. Pair-wise treatment comparisons were performed between 50 mg/kg BO with/without caffeine vs. caffeine alone, 50 mg/kg BO vs. 50 mg/kg SE, and 100 mg/kg BO vs. 100 mg/kg SE.

Dam body weight was analyzed using a mixed model analysis of covariance (ANOCOVA) including terms for treatment, day and interaction, with baseline body weight and number of implants as covariates. Analysis of food consumption was performed with terms for treatment, day and interaction. Within-group correlations were modeled using a heterogeneous first-order autoregressive correlation structure, which allows for correlated differences in variability across days.

A mixed model analysis of variance (ANOVA) was performed to determine the effect of treatment on mean fetal body weight. Within-litter correlations were modeled using a compound symmetric correlation structure.

Analyses of count data for implants, live fetuses and fetuses with skeletal anomalies and of proportion data for sex across litters were performed using generalized linear mixed models. Number of implants, fetuses, split centra and rudimentary ribs across litters were analyzed using Poisson regression. Litter sex proportions across treatment groups were analyzed using logistic regression with dams as blocks.

GLP

Both the dose-finding and the teratology studies were conducted according to 21 CFR Part 58, Good Laboratory Practice for nonclinical Laboratory Studies.

Results

Dose-Finding Study

The results of the dose-finding study are summarized in Tables 1 and 2. At least five rats were pregnant in each group; one animal died during dosing (gavage error), and one animal delivered early (probable missed sperm plug). There were no differences in maternal weight gain, gravid uterine weight, food consumption or in any maternal organ weight. The corrected weight gain was significantly decreased for the 10.0 mg/kg BO group only. The only significant differences in fetal outcome were increased fetal weight, female weight, male weight and the weight of the heart, kidneys and thymus from the pups of the 1.0 mg/kg BO groups. There were no differences in the average numbers of implants, live fetuses or non-live fetuses per litter among the treatment groups. Although the differences were not statistically significant, there were decreases in the numbers of implants/litter and live fetuses/litter and an increase in the number of non-live fetuses/litter at the highest dose of BO (25 mg synephrine/kg body weight). A similar effect was observed at the highest dose of SE (25 mg synephrine/kg body weight), although the differences from control values were less pronounced. These data suggest that higher doses of synephrine might induce more significant embryotoxic effects. No malformations or skeletal anomalies were identified in the dose-finding study.

Teratology Study

Groups of 25–26 females were dosed with one of nine different doses. Sixteen – twenty-two of the animals in each group were actually pregnant (Table 3). A total of six animals died during the study; three of the deaths were due to gavage errors, and the cause of death could

not be determined among the remaining three animals. However, these deaths were distributed among three different treatment groups and did not appear to be related to treatment. No more than two animals delivered prior to GD 21 in each treatment group; these were probably due to missed copulation plugs with the animals being bred again a few days later. All animals that delivered early were removed from the study. There were no differences among the groups in the number of implants/litter. When compared to the vehicle control group, there was a significant decrease in the number of live implants/litter in the 100 mg/kg SE group. Body weights of the maternal animals were significantly lower in the 50 mg/kg BO + caffeine group as well as in the 25 mg/kg caffeine only group relative to the vehicle control group. The lower weights may have been due to decreased food consumption; the 50 mg/kg BO + caffeine group ate significantly less overall than did the control group. The caffeine only group had the second lowest overall food consumption, but this was not statistically different from the control group. There were no differences between treatment and control groups in the mean fetal weight or in the percentage of live pups that were males (Table 4). There were only three fetuses with malformations. These abnormalities included a fetus with bilateral hydronephrosis (50 mg/kg BO + caffeine group), one fetus with exencephaly (100 mg/kg BO group), and one fetus with an unidentified mass in the thoracic cavity (100 mg/kg BO group). None of these malformations appeared to be due to the treatment.

There were numerous skeletal anomalies among all groups (Table 5). Split, or bipartite, thoracic centra were observed in nearly every litter in all treatment groups. At least one centrum was split in most fetuses, but often multiple centra were split in a single fetus. Also, on occasion a lumbar centrum was also split. However, there were no differences between treated and control groups in the frequency of this anomaly. Rudimentary ribs were also common and were present in litters from all treatment groups. There were significantly fewer rudimentary ribs among rats treated with the high dose of SE or with caffeine only relative to the control group; also there were fewer rudimentary ribs among the caffeine only group when compared to the 50 mg/kg BO + caffeine group. There were a few additional skeletal anomalies observed, including split thoracic arches, and one case of fused thoracic ribs.

Discussion

Citrus aurantium extracts are present in a number of dietary supplements marketed for weight loss. Over 73% of respondents in a survey taken in 2002 had reported taking weight loss supplements containing ephedra, caffeine and/or *Citrus aurantium* during the previous year (Blanck et al., 2007). A survey taken in California in 2004 found that 2% of respondents had taken supplements containing bitter orange in the previous year; the authors stated that this incidence might be low due to the presence of ephedra-containing products on the market during a portion of this time (Klontz et al., 2006).

Ephedrine, which is structurally similar to synephrine, has been shown to produce cardiac defects in chicks (Nishikawa et al., 1985b) and rats (Kanai et al., 1986). Nishikawa et al. (1985b) first reported that administration of ephedrine to chick embryos at Hamburger-Hamilton developmental stage 24 (approximately GD 14 in the rat, Butler and Juurlink, 1987) produced an increased incidence of malformations at doses of 1 μ mol and higher. Embryonic survival was not affected at this dose but was decreased at 20 μ mol. Malformations that were produced included cardiac malformations, consisting of ventricular septal defects and conotruncal anomalies, and aortic arch anomalies. Embryos at different stages of development were treated with 14 μ mol ephedrine; aortic arch anomalies occurred across all stages of development as did cardiac defects. They also found that minimally embryotoxic doses of caffeine increased the embryotoxicity of ephedrine (Nishikawa et al.,

1985a), producing cardiac defects such as ventricular septal defects and conotruncal defects as well as aortic arch anomalies. Ventricular septal defects have also been observed among rat embryos when maternal treatment with ephedrine occurred on days 9, 10 or 11 of gestation (Kanai et al., 1986).

When administered to rats daily on GD 6 – 15 by intramuscular injection at 55 or 110 mg/kg, an Italian group found that synephrine produced retardation in skeletal ossification, an increase in brain defects and hemorrhagic injuries (Scrollini et al., 1970, cited in Schardein, 2000). Phenylephrine (*m*-synephrine) was also reported to produce a decrease in birth weight and possible increase in stillbirths in rabbits (Shabanah et al., 1969). However, phenylephrine was reported not to produce defects in rats treated during pregnancy (cited in Schardein, 2000). There is also a single report of an increase in situs inversus in the heart and/or gut of *Xenopus laevis* embryos exposed to octopamine (Toyoizumi et al., 1997).

In the present investigation, the possible developmental toxicity of purified synephrine was compared to an extract that contained approximately 6% synephrine but also contained a variety of other constituents. This latter extract was chosen for analysis since it is commonly used in products marketed for weight loss. The highest dose of synephrine (100 mg/kg SE) significantly decreased the number of live fetuses/litter, but the same synephrine dose (100 mg/kg) using the BO extract did not alter this endpoint. Since the same synephrine dose from the second extract did not alter the number of live fetuses/litter, this suggests that the significant finding with the SE extract may have been a spurious observation. Body weight was significantly decreased for both the caffeine only and the 50 mg/kg BO + caffeine groups. This decrease appeared to be due to a decrease in food consumption, since these two groups had the two lowest average daily food consumption totals. The decrease in food consumption appears to primarily be due to the presence of caffeine, because the 50 mg/kg BO group consumed significantly more food than did the 50 mg/kg BO + caffeine group.

The *Citrus aurantium* dose commonly recommended for humans is about 36–180 mg/day, taken as two to three doses. Assuming a 60–70 kg individual, this would result in a dose of synephrine of up to 3 mg/kg/day. The doses in this study were given as a single bolus dose, rather than as divided doses. Even though the doses in this study were higher than the recommended human dose, they produced no adverse effects on fetal growth and development. The only adverse effect that can be attributed solely to synephrine is the decreased number of live implants observed in the 100 mg/kg SE group; however, the lack of an effect in the 100 mg/kg BO group (which was the same dose of synephrine) suggests that this observation may have been a spurious result.

Caffeine at 25 mg/kg when given by gavage decreased maternal body weight and food consumption in the present study. This finding is in agreement with that of Collins et al. (1981) who observed decreased maternal weight gain at caffeine doses of 6 mg/kg and higher and decreased food consumption during the first trimester of pregnancy at doses of 12 mg/kg and higher. It is perhaps not surprising to find effects on maternal weight after exposure to caffeine since this compound is known to increase metabolic rate (Chou, 1992). Additionally, increases in embryotoxicity and fetal malformations were observed at the two highest doses of caffeine used in the study by Collins and coworkers, 80 and 125 mg/kg, but not at the lower doses of 6, 12 and 40 mg/kg used in that study. The lack of malformations at 25 mg/kg in the present study fits within the observations by Collins et al. (1981).

The lack of abnormal findings in our study compared to that of Scrollini et al. (1970) may be due to either the different route of administration, different strain of rat or different extract used in our study compared to the earlier study. In the study by Scrollini et al. (1970), small groups of pregnant Wistar dams were dosed daily by intramuscular injection from days 7–16

of gestation; whereas, in the present study, we used larger groups of Sprague-Dawley rats that were dosed daily by gavage from days 3–20 of gestation. It is possible that first pass metabolism with the gavage dose would have lead to decreased levels of the compound reaching the fetuses.

A high incidence of skeletal variations was present in this study. These variations were present in both control and treated groups, and there were no statistically significant increases with treatment. Often such variations are thought to be due to maternal stress, and it is unclear if they would have permanent effects (Tyl et al., 2007). The incidence of variations observed in this study is higher than that observed in previous studies from our institution, and the reason for this observation is not clear. It has been some time since a Segment II study was done using our sub-strain of Sprague-Dawley rats, and it is possible that there has been some genetic drift over time.

The results obtained in this study suggest that synephrine present either as a relatively pure compound or in an extract with octopamine, hordenine and tyramine does not produce maternal or developmental toxicity at doses as high as 100 mg synephrine/kg body weight. The addition of a bolus dose of 25 mg of caffeine/kg body weight with a dose of 50 mg of synephrine/kg body weight (using the BO extract) does not increase maternal or developmental toxicity.

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Literature Cited

- Bent S, Padula A, Neuhaus J. Safety and efficacy of *Citrus aurantium* for weight loss. Am J Cardiol. 2004; 94:1359–1361. [PubMed: 15541270]
- Bitsko RH, Reefhuis J, Louik C, Werler M, Feldkamp ML, Waller DK, Frias J, Honein MA. the National Birth Defects Prevention Study. Periconceptional use of weight loss products including ephedra and the association with birth defects. Birth Def Res Pt A. 2008; 82:553–562.
- Blank HM, Serdula MK, Gillespie C, Galuska DA, Sharpe PA, Conway JM, Khan LK, Ainsworth BE. Use of nonprescription dietary supplements for weight loss is common among Americans. J Am Diet Assoc. 2007; 107:441–447. [PubMed: 17324663]
- Broussard CS, Louik C, Honein MA, Mitchell AA. and the National Birth Defects Prevention Study. Herbal use before and during pregnancy. Am J Obstet Gynecol. 2010; 202:443.e1–443.e6. [PubMed: 20035911]
- Butler, H.; Juurlink, BHJ. An Atlas for Staging Mammalian and Chick Embryos. Boca Raton, FL: CRC Press, Inc; 1987. p. 89-101.p. 171-186.
- Colker DM, Kalman DS, Torina GC, Perlis T, Street C. Effects of *Citrus aurantium* extract, caffeine, and St. John's Wort on body fat loss, lipid levels, and mood states in overweight healthy adults. Curr Ther Res. 1999; 60:145–153.
- Collins TFX, Welsh JJ, Black TN, Collins EV. A study of the teratogenic potential of caffeine given by oral intubation to rats. Regul Toxicol Pharmacol. 1981; 1:355–378.
- Evans RL, Siitonen PH. Determination of caffeine and sympathomimetic alkaloids in weight loss supplements by high-performance liquid chromatography. J Chromatogr Sci. 2008; 46:61–67. [PubMed: 18218190]
- Frary CD, Johnson RK, Wang MQ. Food sources and intakes of caffeine in the diets of persons in the United States. J Am Diet Assoc. 2005; 105:110–113. [PubMed: 15635355]

- Fugh-Berman A, Myers A. *Citrus aurantium*, an ingredient of dietary supplements marketed for weight loss: Current status of clinical and basic research. *Exp Biol Med*. 2004; 229:698–704.
- Haller CA, Benowitz NL. Adverse cardiovascular and central nervous system events associated with dietary supplements containing ephedra alkaloids. *New Eng J Med*. 2000; 343:1833–1838. [PubMed: 11117974]
- Kalman DS, Colker CM, Shi Q, Swain MA. Effects of a weight-loss aid in healthy overweight adults: Double-blind, placebo-controlled clinical trial. *Curr Ther Res*. 2000; 61:199–205.
- Kanai T, Nishikawa T, Satoh A, Kajita A. Cardiovascular teratogenicity of ephedrine in rats. *Teratology*. 1986; 34:469.
- Klontz KC, Timbo BB, Street D. Consumption of dietary supplements containing *Citrus aurantium* (bitter orange) – 2004 California Behavioral Risk Factor Surveillance Survey (BRFSS). *Ann Pharmacother*. 2006; 40:1747–1751. [PubMed: 16968826]
- Knight CA, Knight I, Mitchell DC, Zepp JE. Beverage caffeine intake in US consumers and subpopulations of interest: estimates from the Share of Intake Panel survey. *Food Chem Toxicol*. 2004; 42:1923–1930. [PubMed: 15500929]
- LaBorde JB, Pipkin JL Jr, Hinson WG, Anson JF, Sheehan DM, Young JF, Hansen DK. Retinoic acid-induced stress protein synthesis in the mouse. *Life Sci*. 1995; 56:1767–1778. [PubMed: 7739351]
- Louik C, Gardiner P, Kelley K, Mitchell AA. Use of herbal treatments in pregnancy. *Am J Obstet Gynecol*. 2010; 202:439.e1–439.e10. [PubMed: 20452484]
- Marcus DM, Grollman AP. Ephedra-free is not danger-free. *Science*. 2003; 301:1669–1670. [PubMed: 14500960]
- Niemann RA, Gay ML. Determination of ephedrine alkaloids and synephrine in dietary supplements by column-switching cation exchange high-performance liquid chromatography with scanning-wavelength ultraviolet and fluorescence detection. *J Agric Food Chem*. 2003; 51:5630–5638. [PubMed: 12952412]
- Nishikawa T, Bruyere HJ Jr, Gilbert EF, Takagi Y. Potentiating effects of caffeine on the cardiovascular teratogenicity of ephedrine in chick embryos. *Toxicol Lett*. 1985a; 29:65–68. [PubMed: 4082207]
- Nishikawa T, Bruyere HJ Jr, Takagi Y, Gilbert EF, Uno EF. Cardiovascular teratogenicity of ephedrine in chick embryos. *Toxicol Lett*. 1985b; 29:59–63. [PubMed: 4082206]
- NRC. Guide for the care and use of laboratory animals. Washington, DC: Institute for Laboratory Animal Resources; 1996.
- Pillitteri JL, Shiffman S, Rohay JM, Harkins AM, Surton SL, Wadden TA. Use of dietary supplements for weight loss in the United States: Results of a national survey. *Obesity*. 2008; 16:790–796. [PubMed: 18239570]
- SAS Institute Inc. SAS 9.2. Cary, NC:
- Schardein, JL. Chemically-Induced Birth Defects. 3. New York: Marcel Dekker, Inc; 2000. p. 360-366.
- Scrollini F, Sangiovanni M, Torchio P. Effetti teratogenetici comparati della dimetofrina e dell sinefrina, due amine simpaticomimetiche. *Atti della Accademia Medica Lombarda*. 1970; 25:203–207. [PubMed: 5538534]
- Shabanah EH, Tricomi V, Suzrez JR. Fetal environment and its influence on fetal development. *Surg Gynecol Obstet*. 1969; 129:556–564. [PubMed: 5387698]
- Sharpe PA, Granner ML, Conway JM, Ainsworth BE, Dobre M. Availability of weight-loss supplements: Results of an audit of retail outlets in a southeastern city. *J Am Diet Assoc*. 2006; 106:2045–2051. [PubMed: 17126636]
- Shekelle PG, Hardy ML, Morton SC, Maglione M, Mojica WA, Suttrop MJ, Rhodes SL, Jungvig L, Gagne J. Efficacy and safety of ephedra and ephedrine for weight loss and athletic performance. *J Am Med Assoc*. 2003; 289:1537–1545.
- Stuckhardt JH, Poppe SM. Fresh visceral examination of rat and rabbit fetuses used in teratogenicity testing. *Teratog Carcinog Mutagen*. 1984; 4:181–188. [PubMed: 6145223]
- Toyoizumi R, Kobayashi T, Kikukawa A, Oba J, Takeuchi S. Adrenergic neurotransmitters and calcium ionophore-induced situs inversus viscerum in *Xenopus laevis* embryos. *Develop Growth Diff*. 1997; 39:505–514.

Tyl RW, Chernoff N, Rogers JM. Altered axial skeletal development. *Birth Defects Res Pt B*. 2007; 80:451–472.

Ephedrine

Tyramine

Synephrine

Octopamine

Fig 1.

Chemical structure of ephedrine is compared to the structures of synephrine, tyramine and octopamine, alkaloids found in *Citrus aurantium* extract.

Table 1

Maternal parameters from dose-finding study.

	0	1.0 SE ^a	2.5 SE ^a	5.0 SE ^a	10.0 SE ^a	25.0 SE ^a	10.0 BO ^b	25.0 BO ^b
No. Dosed	7	7	7	7	7	7	7	7
No. Pregnant	7	5	6	6	6	6	6	5
No. Non-pregnant or Delivered Early	0	1 ^e	1	1	1	1	0 ^f	2
Wt. Gain (GD 21 – GD 0) ^c	131 ± 21.9	126 ± 8.7	128 ± 15.2	100 ± 30.9	116 ± 28.6	119 ± 33.2	117 ± 23.4	111 ± 28.7
Corrected Wt. Gain (g) ^{c,d}	45.8 ± 11.3	32.0 ± 19.0	36.1 ± 5.9	32.9 ± 10.0	31.8 ± 5.5*	43.5 ± 17.9	29.8 ± 15.6	43.7 ± 10.8
Gravid Uterine Wt. (g) ^c	85.3 ± 25.9	93.7 ± 22.0	92.2 ± 12.5	67.5 ± 34.7	84.7 ± 32.0	75.7 ± 26.9	87.0 ± 12.6	67.5 ± 33.3
Food Consumption (g/day)	20.9 ± 3.8	20.0 ± 2.9	19.5 ± 2.5	19.5 ± 3.7	19.8 ± 2.9	22.3 ± 2.1	20.3 ± 2.3	20.1 ± 3.5
Liver Wt. (g) ^c	13.8 ± 1.47	13.9 ± 2.4	13.1 ± 1.7	12.8 ± 1.6	12.6 ± 2.3	13.9 ± 3.3	13.5 ± 2.8	13.3 ± 2.7
Paired Kidneys Wt. (g) ^c	1.91 ± 0.18	1.97 ± 0.14	1.98 ± 0.16	2.12 ± 0.27	2.10 ± 0.28	2.07 ± 0.30	2.05 ± 0.25	2.06 ± 0.24
Heart Wt. (g) ^c	1.16 ± 0.24	1.31 ± 0.22	1.20 ± 0.17	1.25 ± 0.18	1.21 ± 0.13	1.27 ± 0.14	1.17 ± 0.11	1.17 ± 0.15
Adrenal Glands Wt. (g) ^c	0.12 ± 0.03	0.12 ± 0.03	0.11 ± 0.02	0.11 ± 0.02	0.12 ± 0.02	0.12 ± 0.03	0.12 ± 0.02	0.12 ± 0.01
Brain Wt. (g) ^c	1.99 ± 0.14	1.92 ± 0.12	2.01 ± 0.06	2.02 ± 0.17	1.99 ± 0.12	1.95 ± 0.08	2.00 ± 0.13	1.93 ± 0.10
Thymus Wt. (g) ^c	0.30 ± 0.05	0.31 ± 0.10	0.30 ± 0.03	0.34 ± 0.07	0.31 ± 0.07	0.36 ± 0.08	0.34 ± 0.05	0.34 ± 0.06

^a Doses are in mg/kg/day and were delivered by gavage daily from GD 3 – GD 20. Doses were based on synephrine content and were made up using an extract that was at least 90% synephrine (SE).

^b Doses are in mg/kg/day and were delivered by gavage daily from GD 3 – GD 20. Doses were based on synephrine content and were made up using an extract that was at least 6% synephrine (BO).

^c Data are presented as mean ± SD.

^d Wt. gain – gravid uterine wt.

^e One animal delivered early and was removed from the study.

^f One animal died during dosing.

Table 2

Pregnancy outcomes from dose-finding study.

	0	1.0 SE ^a	2.5 SE ^a	5.0 SE ^a	10.0 SE ^a	25.0 SE ^a	10.0 BO ^b	25.0 BO ^b
No. Treated Animals	7	6	7	7	7	7	6	7
No. Pregnant Animals	7	5	6	6	6	6	6	5
No. Implantations	92	63	83	63	75	68	81	57
Implants/Litter	13.1 ± 2.7	12.6 ± 3.4	13.8 ± 2.0	10.5 ± 4.6	12.5 ± 4.5	11.3 ± 3.6	13.5 ± 1.5	11.4 ± 3.7
No. Live Fetuses	85	63	78	59	72	61	74	46
Live Fetuses/Litter	12.1 ± 3.9	12.6 ± 3.4	13.0 ± 2.3	9.8 ± 4.8	12.0 ± 4.8	10.2 ± 3.8	12.3 ± 2.0	9.2 ± 5.2
Non-Live Fetuses/Litter	1.0 ± 1.5	0.0 ± 0.0	0.8 ± 0.4	0.7 ± 0.8	0.5 ± 0.5	1.2 ± 0.8	1.2 ± 1.2	2.2 ± 2.9
Fetal Wt. ^c	4.96 ± 0.50	5.59 ± 0.19 *	5.17 ± 0.34	4.69 ± 0.78	5.10 ± 0.37	5.23 ± 0.90	5.21 ± 0.41	5.25 ± 0.62
Male Wt. ^c	5.02 ± 0.44	5.70 ± 0.14 *	5.31 ± 0.27	4.92 ± 0.68	5.23 ± 0.36	5.28 ± 0.97	5.29 ± 0.42	5.45 ± 0.51
Female Wt. ^c	4.83 ± 0.71	5.50 ± 0.21 *	5.06 ± 0.39	4.45 ± 1.03	5.00 ± 0.35	5.09 ± 0.85	5.13 ± 0.40	5.13 ± 0.71
Heart Wt. ^c	0.038 ± .004	0.045 ± .005 *	0.043 ± .004	0.038 ± .007	0.041 ± .005	0.042 ± .005	0.041 ± .004	0.041 ± .006
Liver Wt. ^c	0.39 ± 0.07	0.43 ± 0.04	0.38 ± 0.03	0.35 ± 0.07	0.39 ± 0.03	0.40 ± 0.07	0.39 ± 0.06	0.42 ± 0.06
Kidneys Wt. ^c	0.042 ± .007	0.050 ± .004 *	0.046 ± .006	0.038 ± .010	0.045 ± .004	0.045 ± .007	0.044 ± .004	0.048 ± .006
Adrenal Glands Wt. ^c	0.003 ± .001	0.003 ± .001	0.003 ± .001	0.003 ± .001	0.003 ± .001	0.003 ± .001	0.003 ± .001	0.003 ± .001
Thymus Wt. ^c	0.011 ± .002	0.014 ± .002 *	0.012 ± .001	0.011 ± .003	0.012 ± .002	0.013 ± .002	0.011 ± .001	0.012 ± .002

^a Doses are in mg/kg/day and were delivered by gavage daily from GD 3 – GD 20. Doses were based on synephrine content and were made up using an extract that was at least 90% synephrine.^b Doses are in mg/kg/day and were delivered by gavage daily from GD 3 – GD 20. Doses were based on synephrine content and were made up using an extract that was at least 6% synephrine.^c Data are expressed in grams (g) and are presented as mean ± SD.

* Significantly different from vehicle-treated animals.

Table 3

Pregnancy outcomes in teratology study.

	0	10 BO	25 BO	50 BO	100 BO	50 BO + Caffeine	50 SE	100 SE	Caffeine
No. Dosed	25	25	25	26	25	25	25	25	25
No. Pregnant	19	19	16	21	19	18	19	22 ^a	17
No. Non-pregnant	4	5	8	3	4	4	4	1	7
No. Died	1 ^b	0	0	0	1 ^b	1	2(1 ^b)	1	0
Littered Early	1	1	1	2	1	2	0	1	1
No. Implant/Litter	12.9 ± 0.7	12.4 ± 0.7	11.0 ± 1.0	12.5 ± 0.8	12.6 ± 0.7	11.7 ± 0.8	13.7 ± 0.4	10.8 ± 0.6	12.4 ± 0.7
No. Live/Litter	12.9 ± 0.7	12.2 ± 0.7	10.4 ± 1.2	12.4 ± 0.8	12.5 ± 0.7	11.2 ± 0.9	13.5 ± 0.5	9.7 ± 0.9 [*]	11.7 ± 1.0
Body Wt. (g) ^c	317.7 ± 2.2	315.7 ± 2.2	310.8 ± 2.4	315.0 ± 2.1	312.2 ± 2.2	298.6 ± 2.3 [*]	311.8 ± 2.2	310.5 ± 2.1	300.3 ± 2.3 [*]
Corrected Wt. Gain (g) ^d	66.0 ± 3.4	62.6 ± 4.7	61.8 ± 3.9	63.5 ± 3.5	58.9 ± 3.8	43.2 ± 5.6	53.2 ± 8.0	67.5 ± 4.4	48.3 ± 4.2
Food Consumption(g/day; GD3–21) ^e	22.1 ± 0.5	21.7 ± 0.5	20.7 ± 0.5	22.0 ± 0.4 [#]	21.1 ± 0.5	19.4 ± 0.5 [*]	21.4 ± 0.5	21.3 ± 0.4	20.3 ± 0.5

Results are presented as least square mean ± SEM. Doses are in mg synephrine/kg body weight and were delivered by gavage daily from GD 3 – GD 20. BO indicates an extract that was at least 6% synephrine; SE indicates an extract that was at least 90% synephrine.

^a One litter of 3 implantations was completely resorbed.

^b Died due to gavage error.

^c Least square mean body weight was adjusted for GD 0 weight and number of implants.

^d Corrected weight gain = Weight gain (GD 21 wt – GD 0 wt) – gravid uterine wt.

^e Least square mean food consumption data are expressed as grams consumed/day between GD 3 and GD 21.

^{*} Significantly different from vehicle control group.

[#] Significantly different from 50 BO + caffeine group.

Table 4

Fetal outcomes from teratology study.

	0	10 BO	25 BO	50 BO	100 BO	50 BO + Caffeine	50 SE	100 SE	Caffeine
No. Litters	19	19	16	21	19	18	19	21 ^a	17
No. Fetuses	246	232	166	261	238	202	257	214	199
Fetal Wt ^b	5.26 ± 0.08	5.28 ± 0.08	5.27 ± 0.09	5.37 ± 0.08	5.36 ± 0.08	5.17 ± 0.08	5.16 ± 0.08	5.33 ± 0.08	5.24 ± 0.09
Male Wt ^c	5.43 ± 0.09	5.36 ± 0.05	5.47 ± 0.06	5.44 ± 0.05	5.45 ± 0.06	5.32 ± 0.06	5.34 ± 0.06	5.38 ± 0.06	5.28 ± 0.07
Female Wt ^c	5.14 ± 0.07	5.17 ± 0.04	5.07 ± 0.11	5.30 ± 0.05	5.25 ± 0.07	5.02 ± 0.05	5.02 ± 0.05	5.20 ± 0.04	5.18 ± 0.07
% Males	45.1 ± 3.4	53.7 ± 3.3	42.1 ± 5.2	51.9 ± 3.5	53.2 ± 4.0	46.2 ± 4.1	49.6 ± 3.1	46.5 ± 3.8	46.4 ± 4.2
No. Malformed Fetuses ^d	0	0	0	0	2	1	0	0	0

Results are presented as mean ± SEM. Doses are in mg synephrine/kg body weight and were delivered by gavage daily from GD 3 – GD 20. BO indicates an extract that was at least 6% synephrine; SE indicates an extract that was at least 90% synephrine.

^a One litter was completely resorbed and is not included in the data in this table.

^b Least square mean weights are expressed as grams (g).

^c Mean weights (as calculated using formulas in Excel) are expressed as grams (g).

^d Includes gross or visceral abnormalities, but does not include skeletal anomalies. Abnormalities included a fetus with bilateral hydronephrosis (50 mg/kg BO + caffeine), a fetus with exencephaly (100 mg/kg BO) and a fetus with an unidentified mass in the thoracic cavity (100 mg/kg BO).

Table 5

Skeletal anomalies observed in the teratology study.

	0	10 BO	25 BO	50 BO	100 BO	50 BO + Caffeine	50 SE	100 SE	Caffeine
No. Litters	19	19	16	21	19	18	19	21 ^a	17
No. Fetuses	246	232	166	261	238	202	257	214	199
Rud. Ribs									
No. Litters	13	13	7	11	10	12	11	10	7
No. Fetuses	46	34	22	54	33	51	29	17	16
Mean/Litter	2.4 ± 0.6	1.8 ± 0.4	1.4 ± 0.6	2.6 ± 0.7	1.7 ± 0.6	2.8 ± 0.8	1.5 ± 0.5	0.8 ± 0.2 [*]	0.9 ± 0.5 ^{*#}
Split Centra									
No. Litters	19	18	14	20	19	17	18	20	16
No. Fetuses	158	142	96	169	127	149	146	139	130
Mean/Litter	8.3 ± 0.9	7.5 ± 1.0	6.0 ± 1.1	8.0 ± 0.9	6.7 ± 0.8	8.3 ± 1.0	7.7 ± 0.9	6.6 ± 0.8	7.6 ± 0.9
Others	0	2 ^b	3 ^b	1 ^b	0	2 ^b	0	2 ^{b,c}	3 ^b

Results are presented as mean ± SEM. Doses are in mg synephrine/kg body weight and were delivered by gavage daily from GD 3 – GD 20. BO indicates an extract that was at least 6% synephrine; SE indicates an extract that was at least 90% synephrine. Rud. Ribs = Rudimentary Ribs

^a One litter was completely resorbed and is not included in the data in this table.

^b One fetus/litter had a split thoracic arch.

^c One fetus/litter had fused ribs.

^{*} Significantly different from vehicle control group.

[#] Significantly different from 50 BO + caffeine group.